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High Mobility Group Box 1 in Children with Acute Encephalopathy and Other Convulsive Diseases

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Abstract

HMGB1 is known as a representative of danger-associated molecular patterns (DAMPs), which play an important role in triggering inflammatory responses. In order to know the role of high mobility group box 1 (HMGB1) in children with acute encephalopathy and other neurological disorders we analyzed HMGB1 and other cytokines in serum and cerebrospinal fluid. Comparing HMGB1 in Kawasaki disease as a control, serum levels in influenza-associated encephalopathy and West syndrome were significantly lower. However, two cases of influenza-associated encephalopathy having sequelae showed extremely high levels. The levels of HMGB1 in acute encephalopathy and purulent and aseptic meningitis in cerebrospinal fluid were low; however, they were significantly higher than those in West syndrome. Serum HMGB1 levels were correlated with those of interleukin-6 and interleukin-8 in cerebrospinal fluid. HMGB1 is suspected to have a pivotal role in pathophysiology of acute encephalopathy and other neurological disorders.

Keywords: Acute encephalopathy; West syndrome; Influenza; VEGF; IL-6

Introduction

Approximately 1000 children are affected with encephalitis/encephalopathy every year in Japan. Influenza virus is the most frequent cause at approximately one fourth [1-3]. Large amounts of inflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α), are produced in influenza-associated encephalopathy [1,4]. High mobility group box 1 (HMGB1) release occurs actively after cytokine stimulation as well as passively during cell death [5]. HMGB1 is known as a representative of danger-associated molecular patterns (DAMPs), which play an important role in triggering inflammatory responses [6,7]. Recently, anti-HMGB1 antibody has been reported to reduce impairment by cerebral infarction in mice [8]. To clinically investigate the role of HMGB1 in encephalopathy in children, we assayed serum and cerebrospinal fluid (CSF) levels of HMGB1 and other cytokines in encephalopathy and other convulsive diseases.

Materials and Method

Patients

Twelve children with influenza-associated encephalopathy (1 to 9 years old) and 4 patients with febrile convulsions during influenza infection were enrolled. Five children with influenza-associated encephalopathy showed serious sequelae and one child died. Six patients with West syndrome as controls with uncontrolled convulsions and 12 patients with Kawasaki disease as controls with high DAMPs were also included. CSF samples were obtained from 4 patients with acute encephalopathy (2 influenza-associated encephalopathy, one rotavirus-associated encephalopathy, and one Dravet syndrome), 9 patients with epilepsy (West syndrome and neonatal epilepsy), and 7 patients with meningitis (5 purulent and 2 aseptic). Twenty-four serum samples, which were obtained from healthy controls or those without infectious diseases, were also included as controls. All samples were obtained in the acute phase and frozen at -80°C until the assay.

Measurement of HMGB1

The concentrations of HMGB1 in serum and CSF were determined using an HMGB1 ELISA kit (Shino-test Corporation, Japan). The minimal detection limit is 2.5 ng/mL. The reproducibility is within 10%. We assayed 27 cytokines in CSF (IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, Exotaxin, FGF basic, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1β, RANTES, TNF-α and VEGF) by the Bio-Plex suspension array system (Bio-Rad Laboratories, Tokyo, Japan) and the 27 Plex Panel (Bio-Rad Laboratories, Tokyo, Japan). The minimal detection limits of each cytokine are within 0.01 to 100 pg/mL (minimum: IL-4, IL-5, IL-7, IL-13, MIP-1β; maximum: RANTES).

Statistic analysis

Statistical evaluation of the collected data was conducted using the chi-square test and Spearman's correlation coefficient by rank test for independence for significant differences.

Results

Serum HMGB1 levels in patients with influenza-associated encephalopathy and Kawasaki diseases were statistically higher than those of patients with West syndrome (Figure 1). Serum HMGB1 levels in patients with influenza-associated encephalopathy were statistically lowers than those of patients with Kawasaki disease (vasculitis) as a control. However, there were two cases with extremely high levels (>100 ng/mL) of serum HMGB1 in the group of influenza-associated

encephalopathy, similar to those in Kawasaki disease. Serum HMGB1 concentrations in controls all showed less than 81 (mean and standard deviation were 24.7 and 28.7) ng/mL. There was no statistical difference between children with influenza-associated encephalopathy with or without sequelae and febrile seizure (Figure 2). Serum HMGB1 levels in children (n=7) with West syndrome were low (5.5 ± 5.43 ng/mL) except during infection.

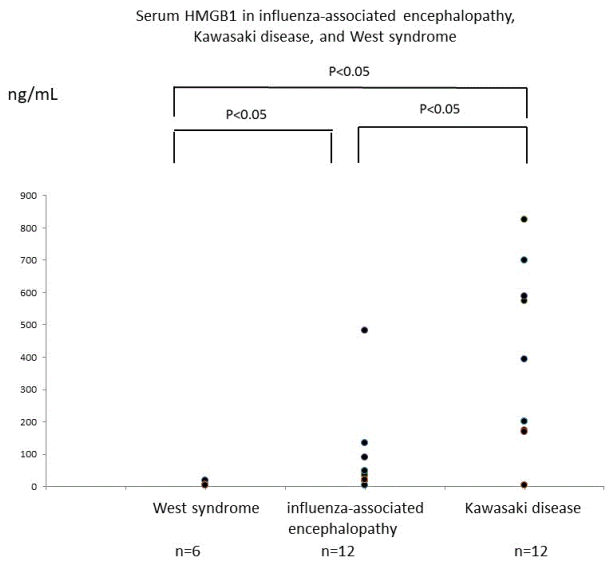

Figure 1: Serum HMGB1 levels in influenza-associated encephalopathy, Kawasaki disease, and West syndrome. There were two cases with high levels of serum HMGB1 in the group with influenza-associated encephalopathy. However, there was no statistical difference in children with West syndrome.

HMGB1 levels in CSF obtained from children with acute encephalopathy (two cases with influenza-associated encephalopathy, one rotavirus-associated encephalopathy, West syndrome, and purulent meningitis) are shown in Figure 3. The CSF HMGB1 levels in acute encephalopathy and purulent meningitis were significantly higher than those in West syndrome.

Serum HMGB1 levels were statistically correlated with only IL-6 and IL-8 in CSF fluid samples obtained from patients with several diseases (Figure 4). There was no significantly correlation between serum HMGB1 and several other cytokines.

Discussion

In systemic vasculitis, including Kawasaki disease, serum HMGB1 levels are higher in patients with active disease compared to healthy controls and the levels are associated with disease severity and mortality. HMGB1 has been suggested to be involved in the pathogenesis of vascular diseases such as systemic vasculitis [9-11]. HMGB1 protein is a late mediator of inflammation or sepsis [12]. However, there are few reports regarding the serum and CSF levels of HMGB1 in children with neurological disorders. Ito et al. reported CSF levels of HMGB1 were not associated with acute encephalopathy due to influenza H1N1 pdm09 [13]. Momonaka et al. measured serum and CSF levels of HMGB1 in patients with influenza-associated encephalopathy and reported that serum HMGB1 levels were significantly higher in patients with poor outcomes compared to those without neurological sequelae. In contrast, there were no differences in CSF HMGB1 levels among all groups [14].

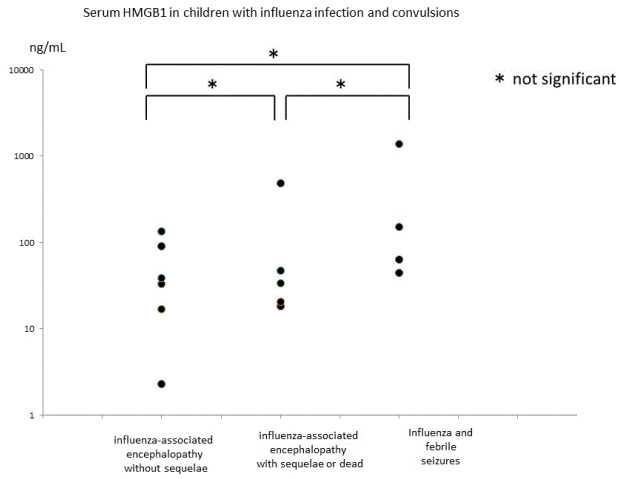

Figure 2: Comparison of serum HMGB1 levels in children with influenza infection is shown. There was no statistical difference in children with influenza-associated encephalopathy with or without sequelae and febrile seizures. All samples were obtained from patients with influenza-associated encephalopathy in the acute phase.

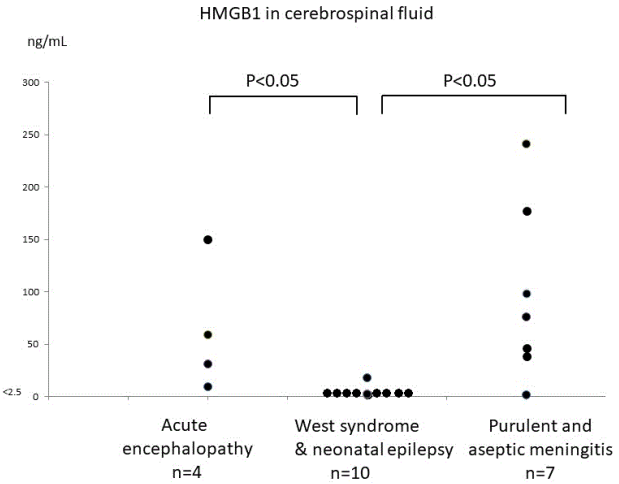

Figure 3: HMGB1 in cerebrospinal fluid obtained from children with acute encephalopathy (two cases with influenza-associated encephalopathy, one rotavirus encephalopathy and Dravet syndrome), West syndrome, and meningitis. The levels in acute encephalopathy and meningitis were significantly higher than those in West syndrome.

In this study, serum levels in influenza-associated encephalopathy were significantly lower compared with the levels in Kawasaki disease

(vasculitis) as control, but higher than those in West syndrome (noninfectious diseases). However, two cases with influenza-associated encephalopathy having sequelae also showed high levels. There is no statistical significant between all groups infected influenza. Influenza virus might induce production of HMGB-1 during infection in some cases. On the other hand, the CSF levels of HMGB1 in acute encephalopathy and purulent and aseptic meningitis were higher than those in West syndrome. Serum HMGB1 levels were significantly correlated with those of IL-6 and IL-8 in CSF. These results show that HMGB1 is suspected to have a pivotal role in pathophysiology of infective acute encephalopathy focused around the neurovascular unit without a direct effect in the brain.

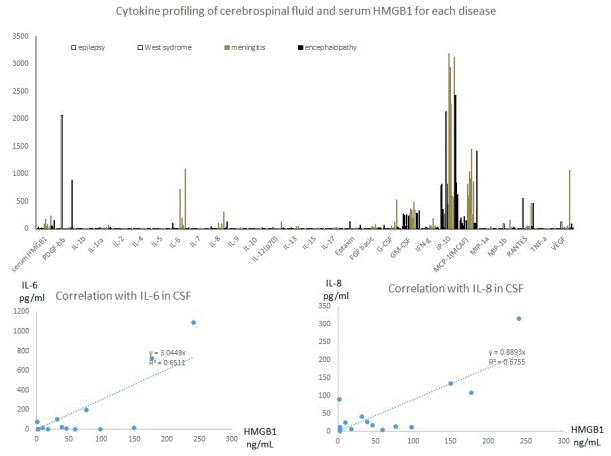

Figure 4: Serum HMGB1 and several CSF cytokines (upper part of figure) and the significant correlations between them (lower part of figure).

Wähämaa et al. reported that extracellular HMGB1 can strongly enhance the cytokine response of TNF, IL-6 and IL-8 evoked by other pro-inflammatory molecules, such as lipopolysaccharide, CpG-DNA, and IL-1β 6). Qiu et al. also reported that HMGB1 promotes MMP-9 upregulation after cerebral ischemia) [15]. Addition of HMGB1 to neuronal and glial cell cultures caused MMP-9 upregulation in a dose- and time-dependent manner [15]. Microinjection of HMGB1 induces upregulation of MMP-9, and the response is independent of TNF-α. Activation of MMP-9 in the central nervous system induces an increase in permeability of the blood-brain barrier (BBB) [16].

One characteristic of influenza-associated encephalopathy is an abrupt onset of unconsciousness with high mortality or critical sequelae. In order to develop a proper treatment for influenza-associated encephalopathy, a specific biomarker of influenza-associated encephalopathy should be found; so far no specific one is known. Several authors also reported high levels of pro-inflammatory cytokines, IL-6 and TNF-α, in CSF; cytokine storms play an important role in the pathophysiology. Production of MMP-916) and free radicals [17,18] and resulting damage to the BBB [19] has been reported to be the pathophysiology in acute encephalopathy. HMGB1 is also a good marker to help predict the outcome in neurological damage [20,21].

Conclusion

Therefore, proper diagnosis of the patients with encephalopathy and early treatment against DAMPs, including HMGB1, might improve sequelae of infective encephalopathy.

Acknowledgement

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Conflict of Interest

The authors declare no conflicts of interest.

Ethical Considerations

The authors declare that all experiments were carried out in compliance with relevant laws and guidelines in accordance with the ethical standards of the Declaration of Helsinki.

References

1. Morishima T, Togashi T, Yokota S, Okuno Y, Miyazaki C, et al. (2002) Encephalitis and encephalopathy associated with an influenza epidemic in Japan. Clin Infect Dis 35: 512-517.
2. Togashi T, Matsuzono Y, Narita M, Morishima T (2004) Influenza-associated acute encephalopathy in Japanese children in 1994-2002. Virus Res103: 75-78.
3. Yoshikawa H, Yamazaki S, Watanabe T, Abe T (2001) Study on influenza-associated encephalitis/encephalopathy in children during the 1997 to 2001 influenza seasons. J Child Neurol 16: 885-890.
4. Ichiyama T, Nishikawa M, Yoshitomi T, Hayashi T, Furukawa S (1998) Tumor necrosis factor-a, interleukin-1ß and interleukin-6 in cerebrospinal fluid from children with prolonged febrile seizures. Neurology 50: 407-411.
5. Sims GP, Rowe DC, Rietdijk ST, Herbst R, Coyle AJ (2010) HMGB1 and RAGE in inflammation and cancer. Annu Rev Immunol 28: 367-388.
6. Wähämaa H, Schierbeck H, Hreggvidsdottir HS, Palmblad K, Aveberger AC, et al. (2011) High mobility group box protein 1 in complex with lipopolysaccharide or IL-1 promotes an increased inflammatory phenotype in synovial fibroblasts. Arthritis Res Ther 13: R136.
7. Hirano H, Yoshioka T, Yunoue S (2012) TLR4, IL-6, IL-18, MyD88 and HMGB1 are highly expressed in intracranial inflammatory lesions and the IgG4/IgG ratio correlates with TLR4 and IL-6. Neuropathology 32: 628-637.
8. Qiu J, Nishimura M, Wang Y (2008) Early release of HMGB-1 from neurons after the onset of brain ischemia. J Cereb Blood Flow Metab 28: 927-938.
9. de Souza AW, Westra J, Limburg PC, Bijl M, Kallenberg CG (2012) HMGB1 in vascular diseases. Autoimmun Rev 11: 909-917.
10. Hoshina T, Kusuhara K, Ikeda K, Mizuno Y, Saito M (2008) High mobility group box 1 (HMGB1) and macrophage migration inhibitory factor (MIF) in Kawasaki disease. Scand J Rheumatol 37: 445-449.
11. Wang C, de Souza AW, Westra J (2015) Emerging role of high mobility group box 1 in ANCA-associated vasculitis. Autoimmun Rev 14: 1057-1065.
12. Chirico V, Lacquaniti A, Salpietro V (2014) High-mobility group box 1 (HMGB1) in childhood: from bench to bedside. Eur J Pediatr 173: 1123-1136.
13. Ito Y, Torii Y, Ohta R (2011) Increased levels of cytokines and high-mobility group box 1 are associated with the development of severe pneumonia, but not acute encephalopathy, in 2009 H1N1 influenza-infected children. Cytokine 56: 180-187.

14. Momonaka H, Hasegawa S, Matsushige T (2014) High mobility group box 1 in patients with 2009 pandemic H1N1 influenza-associated encephalopathy. Brain Dev 36: 484-488.
15. Qiu J, Xu J, Zheng Y (2010) High-mobility group box 1 promotes metalloproteinase-9 upregulation through toll-like receptor 4 after cerebral ischemia. Stroke 41: 2077-2082.
16. Ichiyama T, Morishima T, Kajimoto M, Matsushige T, Matsubara T, et al. (2007) Matrix metalloproteinase-9 and tissue inhibitors of metalloproteinases 1 in influenza-associated encephalopathy. Pediatr Infect Dis J 26: 542-544.
17. Kawashima H, Watanabe Y, Ichiyama T (2002) High concentration of serum NO_x (nitrite/nitrate) obtained from patients with influenza-associated encephalopathy. Pediatr Internat 44: 705-707.
18. Kawashima H, Watan Togashi T (2003) NO_x (nitrite/nitrate) in cerebral spinal fluids obtained from patients with influenza-associated encephalopathy. Neuropediatrics 34: 137-140.
19. Nakai Y, Itoh M, Mizuguchi M (2003) Apoptosis and microglial activation in influenza encephalopathy. Acta Neuropathol 105: 233-239.
20. Asano T, Ichiki K, Koizumi S (2011) High mobility group box 1 in cerebrospinal fluid from several neurological diseases at early time points. Int J Neurosci 121: 480-484.
21. Nakamura T, Yamada S, Yoshioka T (2013) Brain hypothermic therapy dramatically decreases elevated blood concentrations of high mobility group box 1 in neonates with hypoxic-ischemic encephalopathy. Dis Markers 35: 327-330.